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AUTHOR(S):

Shibano, Masaya; Nishida, Shouko; Saito, Yasuko; Kamitakahara, Hiroshi; Takano, Toshiyuki

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Facile synthesis of acyl chitosan isothiocyanates and their application to porphyrin-appended chitosan derivative

Author names and affiliations

Masaya Shibano,[1] Shouko Nishida,[1] Yasuko Saito,[1] Hiroshi Kamitakahara,[1] and Toshiyuki Takano[1]*

[1]Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

***Corresponding author**

Toshiyuki Takano

Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Tel: +81-75-753-6254, Fax: +81-75-753-6300.

E-mail: takatmys@kais.kyoto-u.ac.jp

Abstract

Chitosan (**1**) was reacted with phenylisothiocyanate in 5% AcOH/ H_2O to give *N*-phenylthiocarbamoyl chitosan (**2**) with a degree of substitution (DS) of *N*-phenylthiocarbamoyl groups of 0.86 in 87.1% yield. The following acylation of compound **2** with hexanoyl chloride in the presence of pyridine afforded 3,6-di-*O*-2,3-hexanoyl chitosan isothiocyanate (**4a**) with a DS of the isothiocyanate groups of 0.70 in high yield, unexpectedly. Compound **4a** exhibited high levels of reactivity towards various amines to give the corresponding *N*-thiocarbamoyl chitosan derivatives in high yields. Other acyl (decanoyl (**4b**), myristroyl (**4c**), stearoyl (**4d**), benzoyl (**4e**)) chitosan isothiocyanates were also prepared from chitosan (**1**) in high yields. To evaluate the potential applications of acyl chitosan isothiocyanates, *N*-(triphenylporphynyl)thiocarbamoyl chitosan derivative **6** with a DS of the triphenylporphynyl groups of 0.46 was prepared from compound **4b**. The Langmuir–Blodgett monolayer film of compound **6** gave a good photon-to-electron conversion performance.

Keywords

Acylation, Chitosan, Isothiocyanate, *N*-Phenylthiocarbamoylation, Photocurrent, Porphyrin

1. Introduction

Chitosan is a linear cationic heteropolymer of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues thorough β-1,4 linkages by the deacetylation of chitin which is the second most abundant natural biopolymer in nature, and a most versatile polysaccharide that lends itself to countless chemical and biochemical modifications (Muzzarelli, Tosi, Francescangeli & Muzzarelli 2003; Ravi Kumar et al. 2004; Muzzarelli R.A.A. & Muzarelli, C. 2005; Kurita 2006; Rinaudo 2006; Harish Prashanth & Tharanathan, 2007; Mourya & Inamdar, 2008; Sahoo D., Sahoo S., Morhanty, Sasmal & Nayak, 2009). However, considerable levels of attention have still been focused on the development of the high-value-added utilization for chitosan and its derivatives.

The *N*-substituted thiocarbamoyl chitosan derivatives which was prepared by *N*-thiocarbamoylation of chitosan with isothiocynate compounds are one of the important functional chitosan derivatives. For example, N-acetyl- (Ferkry & Mohamed 2010), *N*-acyl- (Zhong et al. 2008,), *N*-fluoresceinyl- (Qaqish & Amiji, 1999, Ma et al. 2008)), *N*-phenyl- (Baba, Noma, Nakayama & Matsushita, 2002, Monier & Abdel-Latif 2012) thiocarbamoyl chitosan derivatives has been reported as a corrosion inhibitor, an antimicrobial material, a macromolecular

fluorophore, a metal adsorbent, respectively. However, the availability of the commercial isothiocyanate compounds are limited. If chitosan isothiocyanate derivatives are easily synthesized, various amines are available for the syntheses of versatile *N*-substituted thiocarbamoyl chitosan derivatives for new applications. Glucosamine isothiocyanate derivatives can be prepared by the reaction of glucosamine with thiophosgene (Jochims & Seegler, 1965; Fernández-Bolaños, Zafra, López, Robina & Fuentes, 1999), but similar chitosan isothiocyanate derivatives have not been reported in the literature, even though chitosan has an amino group at its C-2 position that could be converted to an isothiocyanate group. The isothiocyanation of amines can be achieved by the reaction of an amine with thiophosgene or carbon disulfide (Mukerjee & Ashare, 1991; Fernández & Millet 1999, Munch, Hansen, Pittelkow, Christensen & Boas, 2008; Sun et al., 2012), although it is important to mention that both of these reagents are highly toxic. With this in mind, the development of a facile and safe synthetic method for the formation of chitosan isothiocyanate derivatives is strongly desired.

We recently reported a facile and safe synthetic method for acyl chitosan isothiocyanates by two reactions, that is, *N*-phenylthiocarbamoylation with

phenylisothiocyanate and acylation with acyl halide or acyl anhydride (Takano & Shibano, 2013). The resulting acyl chitosan isothiocyanates are soluble in common organic solvents and are expected to be useful synthetic intermediates for new functional chitosan derivatives. But, we did not report this procedure in its full detail.

On the other hand, the synthesis of porphyrin-containing chitosan derivatives represents one of several recent proposals for the high-value-added utilization of chitosan, with other examples including the construction of metallotetraphenylporphyrin appended chitosan derivatives (Huang, Guo & Tang, 2007), the use of an Mn (III) deuteroporphyrin-bearing chitosan as catalyst for oxidation reactions (Sun, Hu, Zhao & Liu, 2012), and tetraphenylporphyrin tethered chitosan derivatives for use as nanocarriers for gene delivery (Geware et al., 2013). The LB monolayer films of 6-*O*-porphynyl-2,3-di-*O*-stearoyl cellulose, which is a regioselectively substituted cellulose derivative, have been reported to exhibit high photon-to-electron conversion performances (Sakakibara, Ogawa & Nakatsubo, 2007). The high performance of this material has been attributed to the dense packing of the porphyrin moieties along the cellulose backbone because of the

well-defined and regular structure of the cellulose derivative. The *N*-porphynylthiocarbamoyl chitosan derivatives prepared from the acyl chitosan isothiocyanates could therefore potentially be used as alternative photon-to-electron conversion materials.

This paper provides a detailed account of our new method for the synthesis of acyl chitosan isothiocyanates (Scheme 1). Furthermore, we have described the reactivity of these materials with various amines, and the preparation and evaluation of an LB monolayer film of porphyrin-appended chitosan derivative as one of the examples of the application of the isothiocyanates for the preparation of functional chitosan derivatives.

2. Experimental

2.1. General

Chitosan (DAICHITOSAN 100D (VL), degree of deacetylation 98%) was kindly supplied by Dainichiseika Color & Chemicals Manufacturing Co. (Tokyo, Japan). All of the other chemicals used in the study were purchased from commercial sources and used without further purification. Fourier-transform-infrared (FT-IR) spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer

(Shimadzu, Kyoto, Japan) as KBr pellets (sample 1 mg/ KBr 200 mg). ^{1}H and ^{13}C NMR were recorded on a Varian 500 MHz FT-NMR spectrophotometer (Aglient Technologies, Santa Clara, CA, USA) using tetramethylsilane (TMS) as an internal reference standard in DMSO-d_6 or $CDCl_3$. The standard number of scans in the ^{1}H and ^{13}C NMR measurements were 3500 and 22000, respectively. The chemical shifts (δ) of the NMR spectra have been reported in parts per million (ppm). UV–vis spectra were recorded on a Jasco V-560 UV–vis spectrophotometer (Jasco, Tokyo, Japan).

2.2. Preparation of acyl chitosan isothiocyanate

2.2.1. N-Phenylthiocarbamoylation

Chitosan (**1**, 1.20 g, 7.45 mmol) was dissolved in a 5% (v/v) solution of AcOH in water (30 mL) and the resulting solution was diluted with MeOH (120 mL). Phenyl isothiocyanate (5.34 ml, 44.7 mmol) was then added to the solution, and the resulting mixture was stirred at 35 °C for 24 h, during which time a precipitate formed. The precipitate was filtered, and the filter-cake was washed with MeOH before being collected and suspended in MeOH (300 mL) without drying. The suspension was then stirred at ambient temperature for 30 min and

filtered, and the filter-cake was washed with MeOH. This purification procedure was repeated several times until no absorbance could be detected at 280 nm in the filtrate. The solid product was then dried in vacuo to afford *N*-phenylthiocarbamoyl chitosan (**2**, 1.80 g, 87.1% yield).

*Compound **2*** - DS $_{PhNHCS}$: 0.86 (determined by elemental analysis); FT-IR (KBr): v 3298, 2873, 1660, 1541, 1497, 1373, 1234, 1150, 1065, 898, 746, 692 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.43 (NH), 7.80–7.00 (phenyl-H), 4.69 (H-1), 4.00–3.00 (H-2, H-3, H-4, H-5, H-6a, H-6b) ppm; ^{13}C NMR (DMSO-d_6): δ 182.0 (C=S), 139.5, 129.1, 124.5 (phenyl-C), 102.5 (C-1), 82.0 (C-4), 75.1 (C-5), 73.2 (C-3), 60.6 (C-6), 59.9 (C-2) ppm.

2.2.2. Acylation

*3,6-Di-O-hexanoyl chitosan isothiocyanate (**4a**) (typical method)*

Compound **2** (300 mg, 1.1 mmol) was suspended in a mixture of $CHCl_3$ (6 mL) and pyridine (10 mL), and the resulting suspension was stirred at 35 °C for 24 h. A solution of hexanoyl chloride (1.66 ml, 12.1 mmol) in $CHCl_3$ (4 mL) was then added to the suspension in a drop-wise manner at 0 °C over a period of 10 min. The resulting mixture was then stirred at 1–2 °C for 1 h before being heated at

30 °C for 1 h. The mixture was then heated at 80 °C for 18 h, before being cooled to ambient temperature and poured into MeOH (400 mL). The resulting mixture was stirred at ambient temperature for 2 h and formed a suspension, which was filtered. The filter-cake was then washed with MeOH before being collected and dissolved in a small amount of $CHCl_3$. The resulting solution was added to MeOH (400 mL) in a drop-wise manner to give a suspension, which was filtered. The filter-cake was then washed with MeOH before being collected and dried in vacuo to afford compound **4a** (409 mg).

Compounds **4b**-**4e** were also prepared according to the procedure for compound **4a**. The DS, ^{1}H and ^{13}C NMR and FT-IR data of compounds **4a**-**4e** were summarized in Table 1.

*2.3. Reactivity of hexanoyl chitosan isothiocyanate **4a** with amines*

*3,6-Di-O-hexanoyl-N-phenylthiocarbamoyl chitosan (**5a**) (typical method)*

Aniline (0.23 mL, 2.50 mmol) was added to a solution of compound **4a** (200 mg) in THF (4 mL), and the resulting mixture was stirred at 35 °C for 24 h before being poured into distilled water (400 mL). The resulting precipitate was collected by filtration, and the filter-cake was washed with distilled water before

being collected and dissolved in a small amount of THF. The resulting solution was added to distilled water (400 mL) in a drop-wise manner to give a precipitate, which was collected by filtration. The filter-cake was then washed with distilled water before being collected and dried in vacuo at 40 °C to afford compound **5a** (196 mg).

Compound **4a** was also reacted with *n*-propyl amine and piperidine by the same procedure to give compounds **5b** and **5c**. The DS, ^{1}H and ^{13}C NMR and FT-IR data of compounds **5a**-**5c** were summarized in Table 1.

*2.4. Application of decanoyl chitosan isothiocyanate (**4b**) to the formation of functional chitosan derivatives*

*2.4.1. Preparation of 3,6-di-O-heaxnoyl-N-(p-(10,15,20-triphenyl-5-porphyrinyl)phenyl thiocarbamoyl chitosan (**6**)*

5-(4′-Aminophenyl)-10,15,20-triphenylporphyrin (TPP-NH_2) (29.1 mg), which was prepared according to the method reported by Luguya et al. (2004), was added to a solution of compound **4b** (30 mg) in CH_2Cl_2 (4 mL), and the resulting mixture was stirred at 35 °C for 48 h in the absence of light before being poured

into MeOH (200 mL). The resulting precipitate was collected by centrifugation (3000 ×g, 15 min), and dissolved in a small amount of CH_2Cl_2. The resulting CH_2Cl_2 solution was then added to MeOH (200 mL) in a drop-wise manner to give a precipitate, which was collected by centrifugation (3000 ×g, 15 min). This precipitation/dissolution process was repeated three times. The solid product was then dried in vacuo at 40 °C to afford compound **6** (29 mg).

*Compound **6*** - $DS_{TPPNHCS}$: 0.46 (determined by elemental analysis); FT-IR: v 3415(NH), 2957, 2870, 2047, 1747 (C=O), 1537, 1498, 1377, 1356, 1242, 1167, 1107, 1053, 750, 696 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.80, 8.53, 8.18, 7.97, 7.73, 7.38 (porphyrin-H), 5.40-3.10 (H-1, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.36 (hexanoyl -$OCOCH_2$-), 1.60 (hexanoyl -$OCOCH_2$-CH_2-), 1.26 (hexanoyl -CH_2-), 0.88 (hexanoyl -CH_3), -2.80 (NH of porphyrin) ppm.

*2.4.2. Preparation and evaluation of LB monolayer films of compound **6***

A solution of compound **6** in $CHCl_3$ (0.5 mg/mL) was spread onto a water subphase in a Teflon-coated trough (331 × 100 × 5 mm, USI-3-22T, USI-system, Fukuoka, Japan). Ultrapure water was obtained from a Milli-Q water purification system (Simpli Lab, Merck Japan, Tokyo, Japan) and used for the subphase.

The solvent was evaporated for 30 min and the surface pressure (π)–area (A) isotherms were measured at a constant compression rate of 6 mm/min. The surface pressure was measured using a Wilhelmy-type film balance. The surface pressure was held at 10 mN m^{-1} for 30 min prior to the deposition of the surface monolayer onto the substrates.. The vertical dipping method was used to deposit the surface monolayer onto the substrate with quartz, or an Indium Tin Oxide (ITO) electrode. The downward and upward stroke rates were set at 6 mm/min. The surface pressure was held at 10 mN m^{-1} throughout the deposition process, and the surface temperature was kept at 20 °C for the preparation of the LB monolayer films [i.e., film 6A (on quartz, transfer ratio: downward: 0.00, upward: 1.03), and film 6B (on an ITO electrode, transfer ratio: downward: 0.00, upward: 0.96)]. The photocurrent of film 6B was measured according to a previously reported method (Sakakibara, Ogawa & Nakatsubo, 2007).

3. Results and discussion

3.1. Preparation of acyl chitosan isothiocyanates

The *N*-phenylthiocarbamoylation of chitosan (**1**) was performed according to a

slightly modified version of the method reported by Baba et al (2002). It is noteworthy that the authors of this particular study only reported part of FT-IR data during their characterization of the structure of *N*-phenylthiocarbamoyl chitosan (**2**). In terms of the *N*-phenylthiocarbamoylation of chitosan (**1**) , chitosan was reacted with phenyl isothiocyanate in a mixture of 5% (v/v) AcOH in water and MeOH at 35 °C for 24 h to afford compound **2** in 87.1% yield. The FT-IR spectrum of this compound (Supporting information 1) contained characteristic bands derived from phenylthiocarbamoyl groups at 1541, 1497, 746, and 692 cm^{-1} (Monier & Abdel-Latif, 2012; Shibano, Kamitakahara & Takano, 2013). ^{1}H and ^{13}C NMR analyses of compound **2** revealed signals around 7.0 and 125–135 ppm, which were assigned to the aromatic protons and carbons of the phenylthiocarbamoyl group, respectively. The ^{13}C NMR spectrum of compound **2** also contained a signal at 182.0 ppm, which was assigned to the C=S moiety of the phenylthiocarbamoyl group. The degree of substitution of the phenylthiocarbamoyl groups (DS_{PhNHCS}) in compound **2** was determined to be 0.86 by elemental analysis.

The hexanoylation of compound **2** was performed under typical acylation conditions (i.e., hexanoyl chloride and pyridine at 0 °C for 1 h, 30 °C for 1 h, and

199 80 °C for 18 h sequentially) to give product A in high yield. Analysis of this
200 compound by FT-IR revealed characteristic ester bands at 1747 and 1167 cm^{-1},
201 whereas the band around 3298 cm^{-1} corresponding to the hydroxyl groups and
202 NH moieties of the thioureido groups of compound **2** were absent. Signals
203 characteristic of the hexanoyl groups (Zong, Kimura, Takahashi & Yamane,
204 2000) were also found in the ^{1}H and ^{13}C NMR spectra of product A (Fig.1).
205 Taken together, these results suggested that hexanoylation had proceeded
206 smoothly at both the *O*-3 and *O*-6 positions. In contrast, however, the
207 characteristic bands of the phenylthiocarbamoyl groups at 1541, 1497, 746,
208 and 692 cm^{-1} were not present in the FT-IR spectrum of product A. Furthermore,
209 the aromatic signals of the phenyl moiety of the phenylthiocarbamoyl group
210 around 7.0 and 125–135 ppm had disappeared from the ^{1}H and ^{13}C NMR
211 spectra. These results therefore demonstrated, rather unexpectedly, that the
212 phenylthiocarbamoyl groups were being removed from the chitosan during the
213 hexanoylation process. The FT-IR spectrum of product A also contained a new
214 band at 2047 cm^{-1}, which was consistent with the introduction of isothiocyanate
215 (i.e., -NCS) groups (Shibano, Kamitakahara & Takano, 2013). Furthermore, this
216 band disappeared when product A was reacted with an amine, which provided

217 further evidence that this band related to the presence of NCS groups in
218 product A. NMR analysis of provided further evidence in support of the
219 presence of NCS groups in product A, with a signal consistent with the C=S
220 moiety of the NCS group being observed at 140.8 ppm in the ^{13}C NMR
221 spectrum (Fig.1). Taken together, these data for product A indicated that this
222 material was not 3,6-di-*O*-hexanoyl *N*-(hexanoyl)phenylthiocarbamoyl chitosan
223 (**3a**) as expected, but 3,6-di-*O*-hexanoyl chitosan isothiocyanate (**4a**). The
224 DS_{NCS} of compound **4a** was determined to be 0.74 by elemental analysis.

225 Fig.2 shows the FT-IR spectra of the products resulting from the
226 hexanoylation of compound **2** at various time points during the 80 °C heating
227 stage of the reaction. The results of this analysis revealed that the characteristic
228 bands of the ester and amide groups at 1747 and 1167 cm^{-1} and 1678 cm^{-1},
229 respectively, (Mohamed & Abd El-Ghany, 2012) appeared rapidly after only 1 h,
230 whereas the bands attributed to the hydroxyl and thiourea groups at 3298 cm^{-1}
231 were reduced significantly. These changes in the FT-IR spectra indicated that
232 the *O*-hexanoylation of the 3-OH and 6-OH positions had proceeded smoothly,
233 as well as the *N*-hexanoylation of the phenylthiocarbamoyl groups. The ^{1}H
234 NMR spectrum of the product after 1 h, however, showed that the

235 *O*-hexanoylation process had not proceeded to completion (data not shown).
236 The FT-IR spectrum of the product after 1 h of the 80 °C heating stage
237 contained a small band at 2047 cm^{-1} for the NCS groups, which suggested that
238 the *N*-phenylthiocarbamoyl groups were beginning to degrade during the first
239 hour of this heating stage. As the reaction increased, there was an increase in
240 the intensity of the band at 2047 cm^{-1}, whereas the intensities of the bands at
241 1678, 1541, 1497, 746, and 692 cm^{-1} decreased. After 18 h, the bands at 1678,
242 1541, 1497, 746, and 692 cm^{-1} were disappeared completely, suggesting that
243 the *N*-(hexanoyl)phenylthiocarbamoyl groups had been fully degraded.

244 *N,N′*-Disubstituted thioureas are known to decompose to the
245 corresponding amines and isothiocyanates when they are heated (Mukerjee &
246 Ashare, 1991). For example, the pyrolysis of *N*-benzoyl-*N′*-phenylthiourea at
247 180 °C was reported to afford phenyl isothiocyanate in high yield (Rajappa,
248 Rajagopalan, Sreenivasan & Kanal, 1979). Based on these reports and the
249 FT-IR spectra shown in Fig.2, we have proposed a mechanism for this
250 transformation which is shown in Fig.3. Briefly, the phenylthiocarbamoyl groups
251 of compound **2** would be converted to the *N,N*-(hexanoyl)phenylthiocarbamoyl
252 groups during *O*-hexanoylation process. The

253 *N,N*-(hexanoyl)phenylthiocarbamoyl groups would then be degraded by the
254 abstraction of a proton by pyridine, which would resulted in the formation of the
255 NCS groups.

256 To evaluate the versatility of this method, we investigated the use of
257 several other acylating agents for the acylation of compound **2** (i.e.,
258 dodecanoylation, myristoylation, stearoylation, and benzoylation) under the
259 same conditions as those used for the hexanoylation reaction, which afforded
260 compounds **4b**–**e** in high yields. The FT-IR spectra of compounds **4a–d**
261 revealed that the characteristic bands of the phenylthiocarbamoyl groups at
262 1541, 1497, 746, and 694 cm^{-1} had disappeared, and that the characteristic
263 bands of the NCS and ester groups had appeared around 2047 cm^{-1}, and
264 around 1748 and 1159 cm^{-1}, respectively (Supporting information 1). These
265 results indicated that the isothiocyanation reaction had proceeded in all cases
266 regardless of the acyl group used in the acylation reaction. The DS_{NCS} values of
267 compounds **4b–d** and **4e** were determined to be 0.70 and 0.56, respectively, by
268 elemental analysis. The solubility of compound **2**, as well as those of
269 compounds **4a–e** are summarized in Table 2. The acyl chitosan isothiocyanates
270 **4a–e** were found to be soluble in a range of common solvents, including THF,

271 $CHCl_3$, and CH_2Cl_2. Interestingly, however, compounds **4a–e** became insoluble
272 in these solvents when they were stored as drying solids at ambient
273 temperature for more than several days. Subsequent testing of the insoluble
274 solid materials by FT-IR spectroscopy revealed that they were analytically
275 identical to the initial solids (data not shown). Similar insolublization behavior
276 has also been observed for compound **2** and 6-isothiocyanato cellulose
277 derivatives (Shibano, Kamitakahara & Takano, 2013).

278 The *N*-phenylthiocarbamoylation of chitosan with phenyl isothiocyanate,
279 followed by acylation with acyl chloride under basic conditions (i.e., in the
280 presence of pyridine) has therefore been demonstrated as effective process for
281 the preparation of acyl chitosan isothiocyanates. Furthermore, this method
282 allows for the use of harmful reagents such as thiophosgene to be avoided. In
283 many ways, our newly developed method represents a trans-isothiocyanation
284 reaction from a phenyl isothiocyanate to an acyl chitosan isothiocyanates in two
285 reactions.

286

287 *3.2. Reactivity of hexanoyl chitosan isothiocyanate **4a** with amines*

288 Sugar isothiocyanates are known to react readily with amines to form thioureas

289 (Pérez, Mellet, Fuentes & Fernández, 2000). To confirm it, we proceeded to
290 investigate the reactivity of the acyl chitosan isothiocyanates towards a variety
291 of amines. When compound **4a** was reacted with aniline (aromatic amine) in
292 THF at 35 °C for 24 h, compound **5a** was formed in high yield. The FT-IR
293 spectrum of compound **5a** contained the characteristic bands of the
294 phenylthiocarbamoyl groups at 1537, 1497, 750, and 696 cm^{-1}, whereas the
295 characteristic NCS band at 2047 cm^{-1} had disappeared. Furthermore, the ^{13}C
296 NMR spectrum of compound **5a** contained a new signal at 181.0 ppm for the
297 C=S moiety of the newly formed phenylthiocarbamoyl group, which indicated
298 that the reaction of compound **4a** with aniline had proceeded smoothly.
299 Compound **4a** was also reacted with propyl amine (aliphatic primary amine) and
300 piperidine (aliphatic secondary amine) under the same conditions to give the
301 corresponding compounds **5b** and **5c** in high yields, respectively. These results
302 demonstrated that the acyl chitosan isothiocyanates were highly reactive
303 towards amino compounds, and could therefore be used as intermediates for
304 the synthesis of *N*-thiocarbamoyl chitosan derivatives.
305
306 *3.3. Formation of a functional chitosan derivative from decanoyl chitosan*

307 *isothiocyanate **4b***

308 The acyl chitosan isothiocyanate **4b** was converted to the porphyrin-appended
309 chitosan derivative **6** to demonstrate the potential application of these
310 compounds for the formation of functional chitosan derivatives. Compound **4b**
311 was reacted with TPP-NH_2 in CH_2Cl_2 at 35 °C for 48 h to give compounds **6** in
312 high yield. The FT-IR spectrum of compound **6** contained the characteristic
313 bands of decanoyl chitosan at 2926, 2854, 1744, 1155, 1111, and 1055 cm^{-1},
314 as well as those from the porphyrin at 3415, 1597, 1468, 1350, 1178, 966, 800,
315 732, and 702 cm^{-1}, and those from the thiourea groups at 1547 cm^{-1} (Fig.4). It
316 is noteworthy that a small band corresponding to the NCS group was detected
317 at 2039 cm^{-1} in FT-IR spectrum of compound **6**, which indicated that the
318 reaction with TPP-NH_2 had not proceeded to completion. The ^1H NMR
319 spectrum of compound **6** contained signals from the aromatic protons of the
320 porphyrin ring in the range 7.2–9.0 ppm, as well as the pyrrole-NH proton of the
321 porphyrin ring at –2.80 ppm (Luguya et al. 2004) (Supporting information 2).
322 The UV-vis spectrum of compound **6** in chloroform contained a Soret band in
323 the range of 350–450 nm (Supporting information 3). These results clearly
324 indicated that compound **6** was the expected porphyrin-appended chitosan

derivative. The $DS_{TPPNHCS}$ value of compound **6** was determined to be 0.46 by elemental analysis. This medium DS value was attributed to the steric hindrance of the porphyrin groups, because a similar effect was also observed in the corresponding porphyrin-appended cellulose derivative (Sakakibara, Ogawa & Nakatsubo, 2007).

LB monolayer films of compound **6** were prepared on quartz (film 6A) and on an ITO electrode (film 6B) using the vertical dipping method with surface a pressure of 5 mN/m, which was decided based on the surface pressure (π)-area (A) isotherm of compound **6** at the air-water interface at 20 °C (Supporting information 4). In both cases, the monolayer film on the water was not transferred during the first down stroke, but was transferred during the second up stroke with a transfer ratio of almost 1.0, which indicated that films 6A and 6B were Z-type LB films. Film 6A was subjected to UV-vis analysis, whereas 6B was evaluated in terms of its photocurrent generation performance. The UV-vis spectrum of film 6A (solid state) had a similar profile to that of compound **6** in chloroform (solution state), which suggested that the monolayer had been successfully transferred. Fig. 5i shows the photoelectrochemical response of film 6B with illumination at 420 nm. The photocurrent was

generated quickly when film 6B was illuminated. Fig. 5ii shows the action spectrum of film 6B (circles) and the UV-vis spectrum of film 6A (solid line). The patterns of these two spectra were very similar, which suggested that the porphyrin moieties of compound **6** were effectively behaving as photoactive species for the generation of the photocurrent, based on the absorption spectrum. The photocurrent density (i.e., photocurrent per unit area of a working electrode) for film 6B at 420 nm was 236 μA/cm^2. This value was lower than that of an LB monolayer film constructed from a porphyrin-appended cellulose derivative, which had a $DS_{porphyrine}$ value of 0.64 (Sakakibara, Ogawa & Nakatsubo, 2007), and could therefore have been lower because of the lower DS_{TPPNH2} value of compound **6**. Taken together, these results suggest that compound **6** could be used as an effective alternative photon-to-electron conversion material in biomaterial-based solar cells.

4. Conclusion

A facile new method has been developed for the synthesis of for the preparation of acyl chitosan isothiocyanates based on the *N*-phenylthiocarbamoylation of chitosan followed by acylation of the resulting thiocarbamoylated material under

basic conditions. Surprisingly, the formation of the NCS groups of the acyl chitosan isothiocyanates occurred as a consequence of the degradation of the *N*,*N*-(acyl)phenylthiocarbamoyl groups under the basic conditions required of the acylation reaction. A similar outcome was observed when the acylation reaction was conducted with acyl anhydride species under basic conditions, and the details of this alternative method will be published in our next paper.

The acyl chitosan isothiocyanates exhibited a high level of reactivity towards amines to afford the corresponding *N*-thiocarbamoyl chitosan derivatives, which suggested that various functional amines could be used to for the functionalization of chitosan. A porphyrin-appended chitosan derivative (**6**) was also prepared to evaluate the application of these acyl chitosan isothiocyanates to the synthesis of functional materials. The LB monolayer film of compound **6** gave a good photon-to-electron conversion performance, which suggested that compound **6** could be used as a promising photon-to-electron conversion material. Taken together, the results of this study demonstrate that our new method can be used to be provide rapid access to a range of acyl chitosan isothiocyanates, which have the potential to become useful intermediates for the construction of functional chitosan derivatives.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org,/10.106/j.carbpol. .

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479

Legends of Figures & Tables

(Figures & Table)

Scheme 1 Preparation of *N*-substituted thiocarbamoyl chitosan derivatives (**5a–c** and **6**) via the corresponding acyl chitosan isothiocyanates (**4a–e**).

Figure 1. ^{1}H and ^{13}C NMR spectra of product A (Compound **4a**).

Figure 2. FT-IR spectra of the products during the 80 °C heating stage for the hexanoylation of compound **2** (normalized at 1379 cm^{-1}).

Figure 3. Proposed reaction mechanism for the formation of isothiocyanate groups.

Figure 4. FT-IR spectra of compounds **4b** (A); **6** (B); and TPP-NH_2 (C).

Figure 5. (i) Photoelectrochemical response of the LB monolayer film 6B with illumination at 420 nm; (ii) Action spectrum of film 6B (circles); UV-vis spectrum of film 6A (solid line).

Table 1. Data of compounds **4a-e** and **5a-c**

Table 2. Solubility of chitosan derivatives **2** and **4a–e**

(Supporting information)

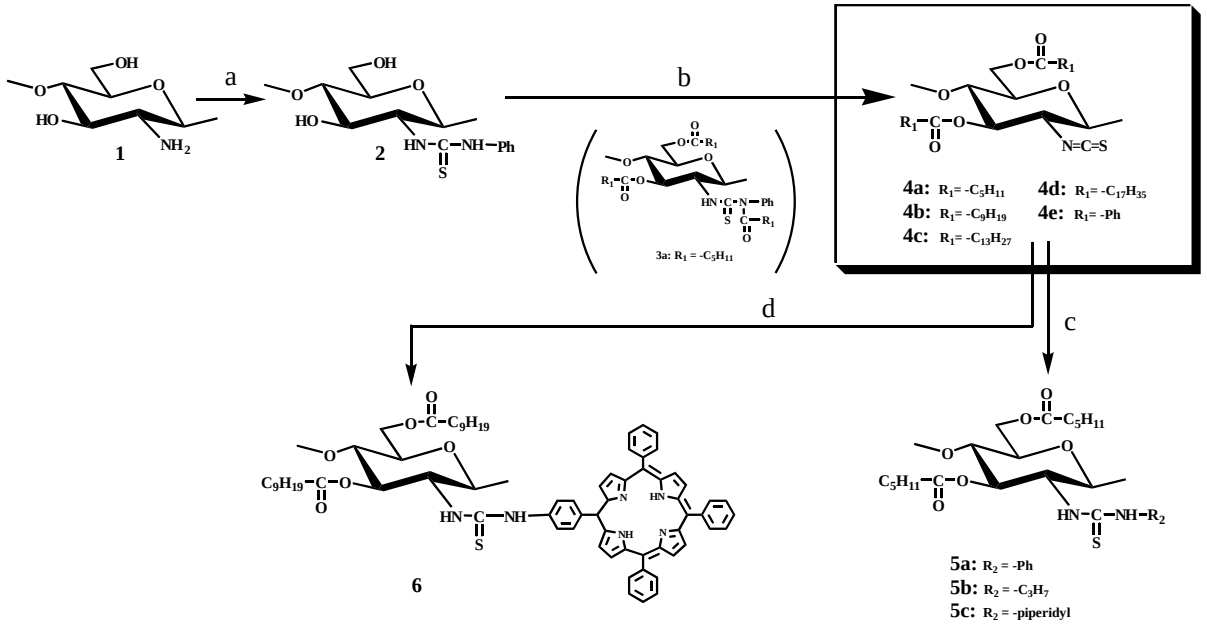
Supporting information 1
FT-IR spectra of compounds **1**, **2**, **4a–e** and **5a–c.**

Supporting information 2
^{1}H NMR spectrum of compound **6**.

Supporting information 3
UV-vis spectra of compound **6** in $CHCl_3$ (solid line) and the LB monolayer film 6A (dashed line) (normalized at 424 nm).

Supporting information 4

Surface pressure (π)-area (A) isotherm of compound **6**



a) Ph-N=C=S/ 5%-AcOH/H_2O/ MeOH, 30ºC, 48 h ; b) Acyl chloride/ Pyridine / $CHCl_3$, 1ºC, 1h → 30ºC, 1 h→ 80ºC, 18 h
c) Amine/ THF, 35ºC, 48 h; d) TPP-NH_2/ CH_2Cl_2, 35ºC, 48 h

Scheme 1 Preparation of *N*-substituted thiocarbamoyl chitosan derivatives (**5a-c** and **6**) via acyl chitosan isothiocyanates (**4a-e**)

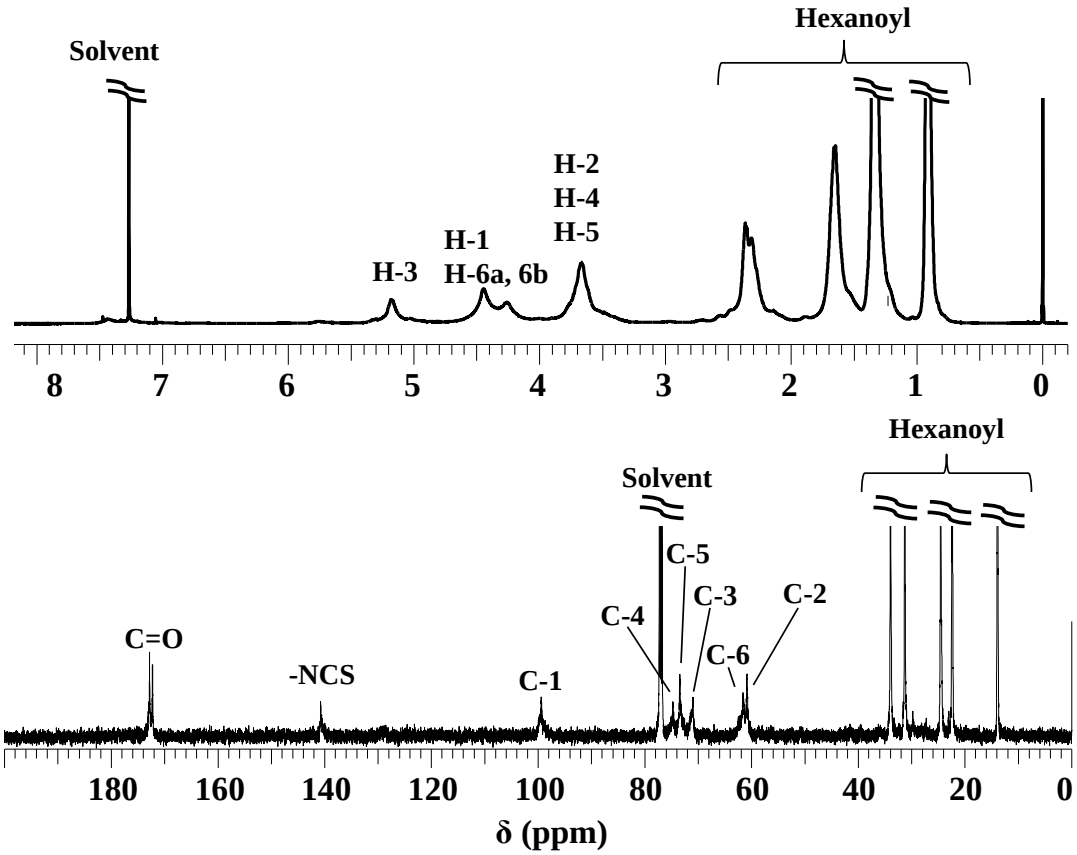


Figure 1. ^{1}H- and ^{13}C-NMR spectra of product A (Compound **4a**)

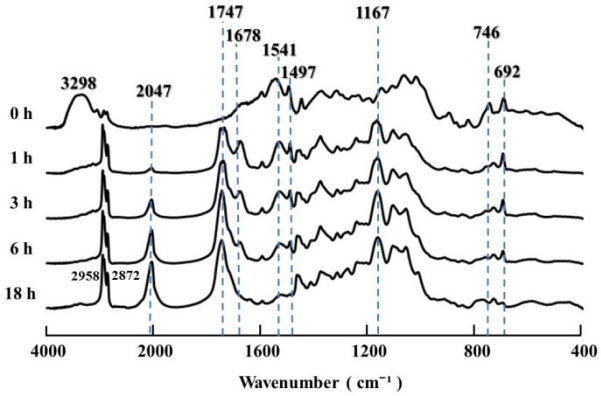


Figure 2. FT-IR spectra of the products at the 80ºC stage in hexanoylation of compound **2** (normalized at 1379 cm^{-1})

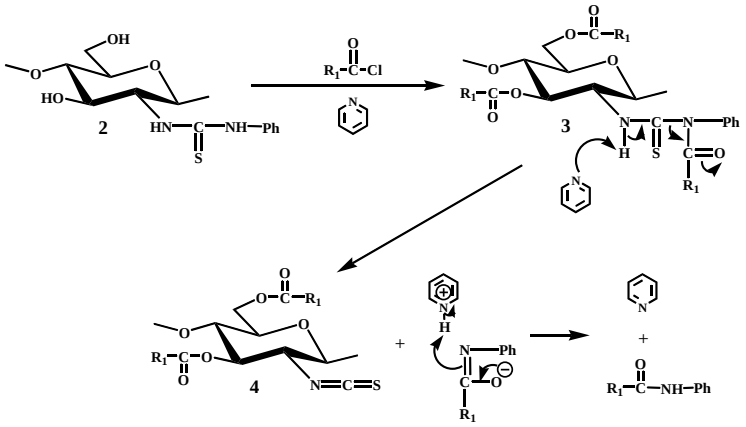


Figure 3. Proposed reaction mechanism for the formation of isothiocyanate groups

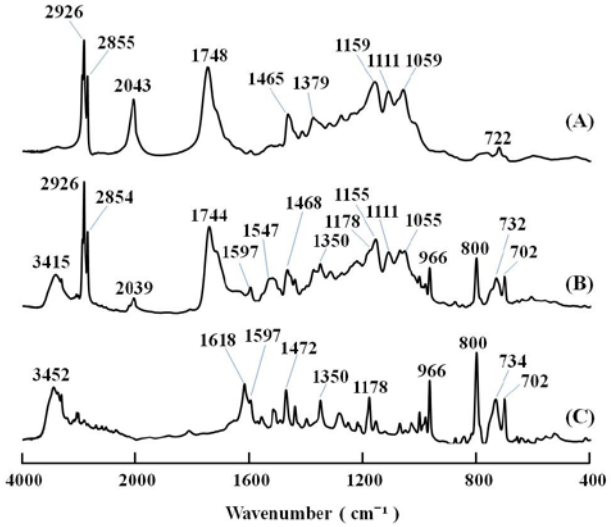


Figure 4. FT-IR spectra of compounds **4b** (A); **6** (B); TPP-NH_2 (C)

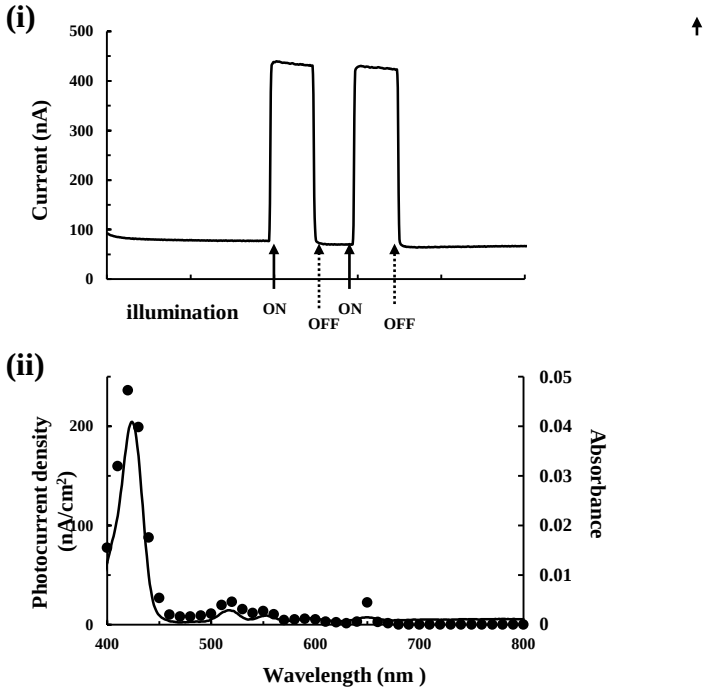


Figure 5. (i) Photoelectrochemical response of the LB monolayer film 6B with illumination at 420 nm; (ii) Action spectrum of film 6B (circles); UV-vis spectrum of film 6A (solid line) .

Table 1 Data of compounds **4a-e** and **5a-c**

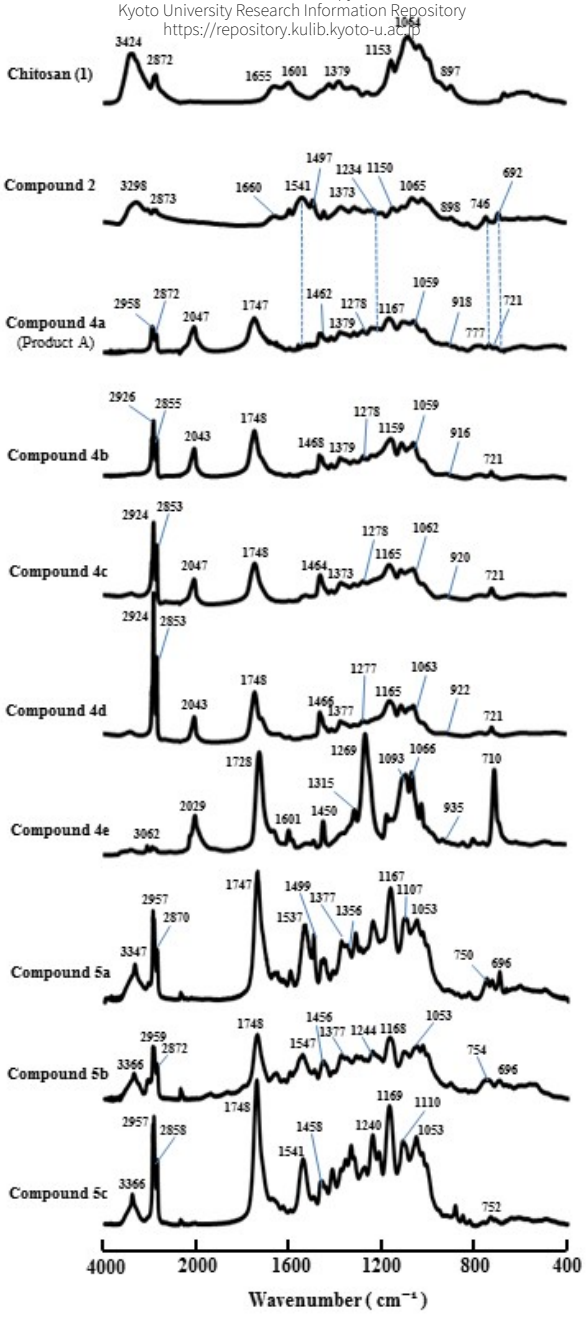
Compound (Acyl group)	**4a** (hexanoyl)	**4b** (decanoyl)	**4c** (myristroyl)	**4d** (stearoyl)	**4e** (benzoyl)	**5a** (hexanoyl)	**5b** (hexanoyl)	**5c** (hexanoyl)
DS*	0.74 NCS	0.70 NCS	0.70 NCS	0.70 NCS	0.56 NCS	0.68 PhNHCS-	0.68 PrNHCS-	0.64 PiperidylNHCS-
^{1}H NMR (in $CDCl_3$) (ppm)								
H-3	5.18	5.17	5.17	5.17	5.22	5.03	5.10	5.01
H-1, H-6a	4.45	4.44	4.45	4.45	4.28	4.80-4.00	4.50-4.00	4.65-4.00
H-6b	4.26	4.26	4.27	4.26	4.09			
H-2, H-4, H-5	3.67	3.67	3.67	3.67	3.67	4.00-3.40	4.00-3.40	4.00-3.40
acyl -OCO*CH*$_2$-	2.37	2.35	2.38	2.35	-	2.30	2.34	2.34
acyl -OCOCH$_2$-*CH*$_2$-	1.65	1.64	1.64	1.60	-	1.58	1.62	1.61
acyl -*CH*$_2$-	1.34	1.27	1.26	1.26	-	1.29	1.32	1.32
acyl -*CH*$_3$	0.91	0.88	0.88	0.88	-	0.88	0.89	0.88
Others	-	-	-	-	8.17-7.67, 7.64-6.90 (benzoyl aromatic-H)	7.60-7.06 (phenyl aromatic-H)	2.34, 1.32, 0.89 (propyl-H)	2.60-2.20, 1.51 (piperidyl-H)
^{13}C NMR (in $CDCl_3$) (ppm)								
C=S	-	-	-	-	-	181.0	183.1	180.9
C=O	172.8, 172.3	172.8, 172.3	172.8, 172.2	172.8, 172.2	164.9, 164.8	173.5, 173.4	173.5, 173.4	173.7, 173.6
NCS	140.8	140.8	140.8	140.8	140.9	-	-	-
C-1	99.5	99.5	99.6	99.6	99.5	101.0	101.3	102.3
C-2	60.9	60.8	60.8	60.9	60.9	58.3	58.9	58.9
C-3	71.0	71.3	71.7	71.5	71.1	71.2	71.5	73.2
C-4	74.7	74.7	74.6	74.6	74.7	74.7	75.3	77.5
C-5	73.4	73.4	73.4	73.4	73.4	73.2	72.5	73.4
C-6	61.6	61.6	61.6	61.6	62.1	62.6	63.0	62.8
acyl -C	33.9, 31.3, 24.5	34.0, 31.8, 29.5	34.0, 31.8, 29.4	34.1, 31.9, 29.4	-	33.9, 31.3, 24.5	34.0, 31.3, 24.5	34.0, 31.3, 24.5
	22.4, 13.9	24.9, 22.7, 14.1	24.9, 22.7, 14.1	24.9, 22.7, 14.1	-	22.3, 13.9	22.3, 14.0	22.4, 13.9
Others	-	-	-	-	133.4, 129.4, 128.6 (benzoyl aromatic-C)	132.6, 129.9, 124.8 (phenyl aromatic-C)	46.8, 24.9, 11.4 (propyl-C)	49.2, 25.6, 22.5 (piperidyl-C)
FT-IR (cm^{-1})								
	2958, 2872, 2047	2926, 2855, 2043	2924, 2853, 2047	2924, 2853, 2043	3062, 2029, 1728	3347, 2957, 2870	3366, 2959, 2872	3399, 2934, 2856
	1747, 1462, 1379	1748, 1468, 1379	1748, 1464, 1373	1748, 1466, 1377	1601, 1450, 1315	1747, 1537, 1499	1748, 1547, 1456	1748, 1541, 1495
	1278, 1167, 1059	1279, 1159, 1059	1278, 1165, 1062	1278, 1163, 1061	1269, 1093, 1066	1377, 1356, 1242	1377, 1356, 1244	1377, 1358, 1240
	918, 777, 721	916, 721	920, 721	922, 721	935, 710	1167, 1107, 1053	1168, 1110, 1053	1169, 1110, 1053
						750, 696	754, 696	752

* The DS (degree of substitution) were determined by elemnatary analyses.

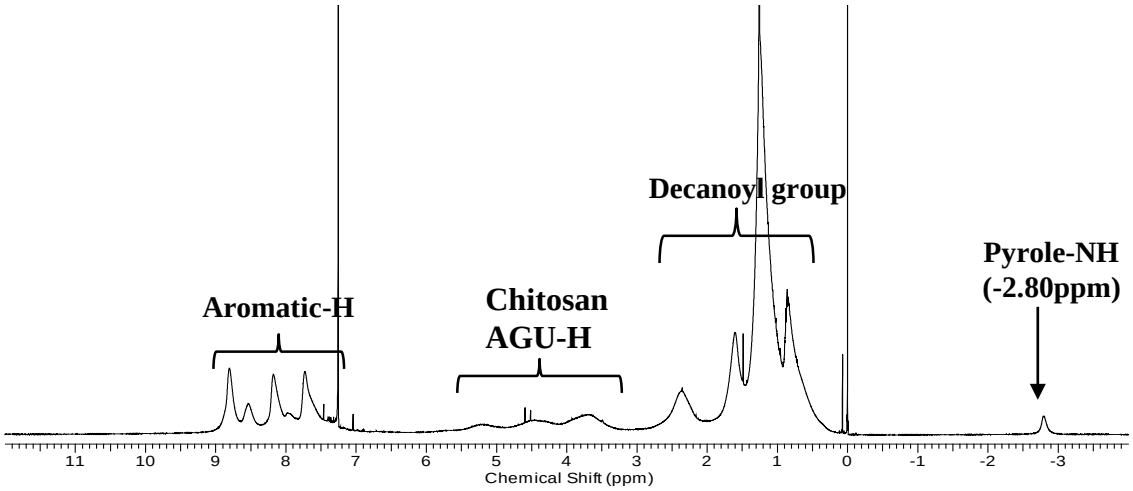
Table 2: Solublity of chitosan derivatives **2** and **4a-4e**

Solvents	δ	Compound					
		2	**4a**	**4b**	**4c**	**4d**	**4e**
THF	9.1	×	○	○	○	○	○
Chloroform	9.3	×	○	○	○	○	○
Acetone	9.4	×	○	△	△	×	○
Dichloromethane	9.6	×	○	○	○	○	○
Dioxane	9.8	×	○	○	△	△	○
DMF	11.5	○	○	△	△	×	○
DMSO	12.8	○	△	△	×	×	○
Methanol	12.9	×	×	×	×	×	×
Water	21.0	×	×	×	×	×	×

δ: Solubility parameter; ○: Soluble, △: Partially soluble, ×: Insoluble

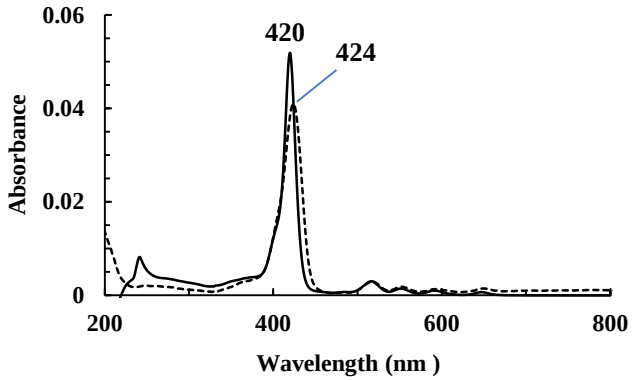


Supporting information 1 FT-IR spectra of compounds **1**, **2**, **4a-e** and **5a-c**



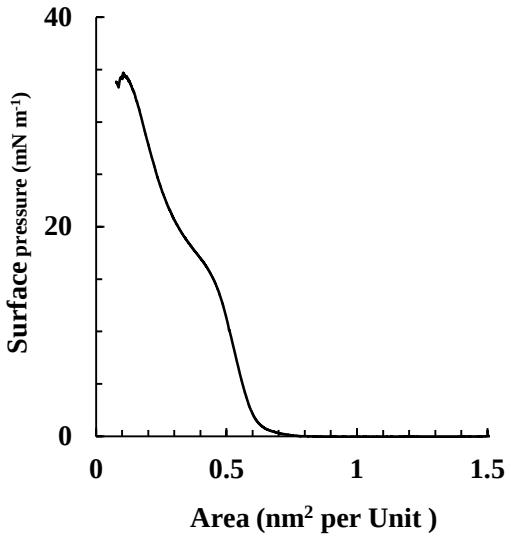
Supporting information 2

^{1}H-NMR spectrum of compound **6**.



Supporting information 3

UV-vis spectra of compound **6** in $CHCl_3$ (solid line) and the LB monolayer film 6A (dashed line) (normalized at 424 nm).



Supporting information 4
Surface pressure (π)-area (A) isotherm of compound **6**